United States Environmental Protection Agency

ToxCast™ One Step in the NRC Vision of 21st Century Toxicology

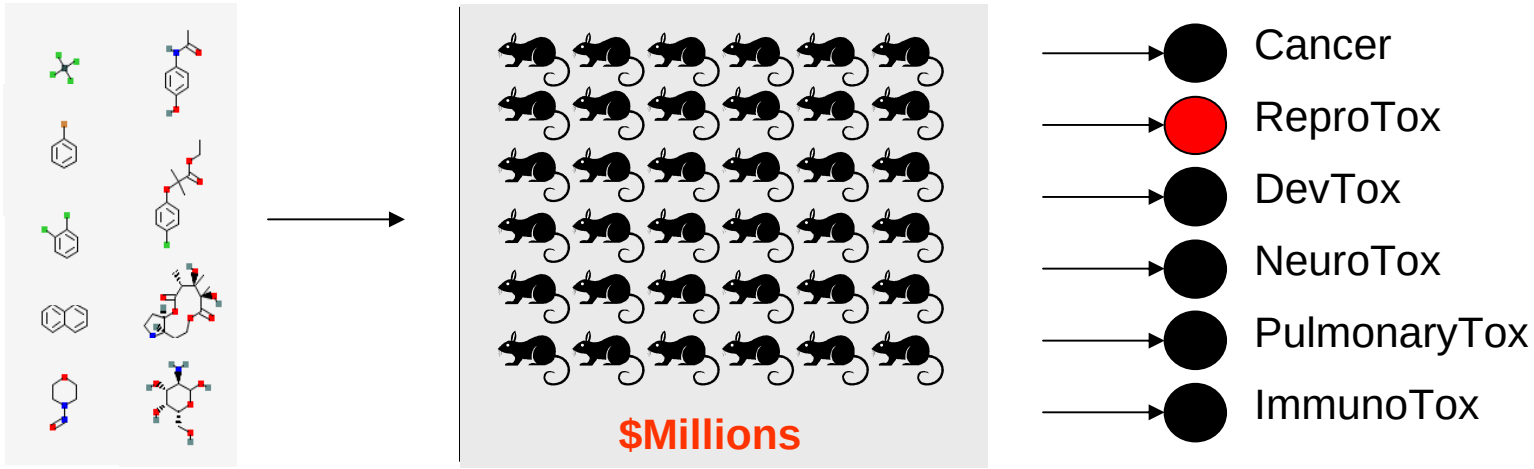
ICCA-LRI Workshop on 21st Century Approaches
Amsterdam, The Netherlands

Office of Research and Development
National Center for Computational Toxicology

June 16, 2008

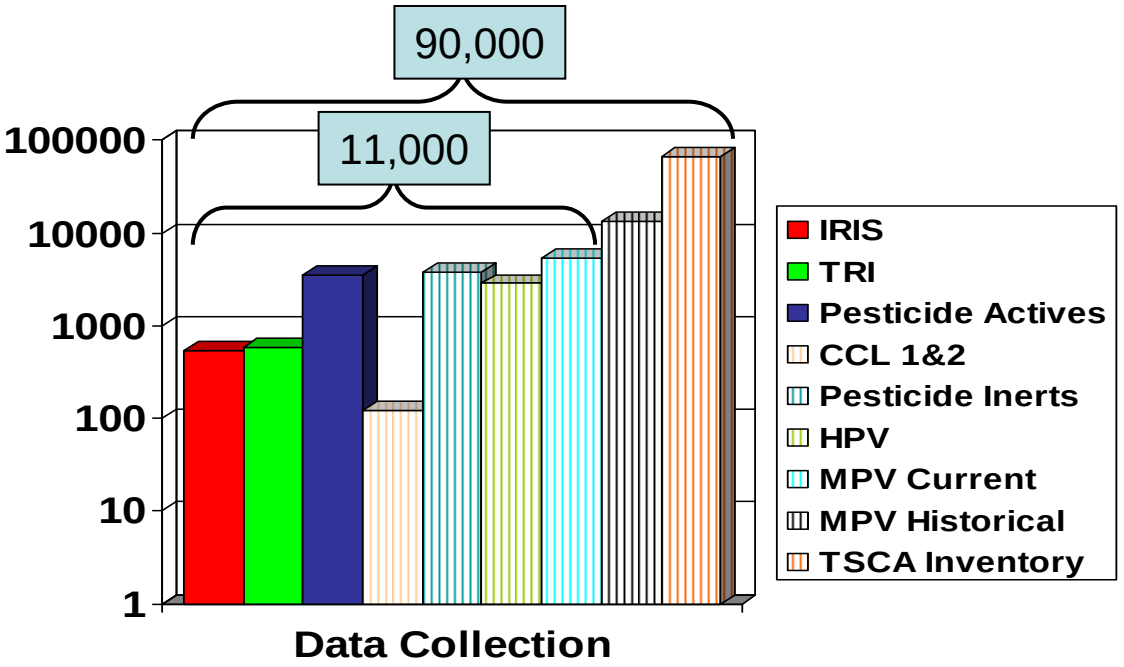
Current Approach for Toxicity Testing

in vivo testing

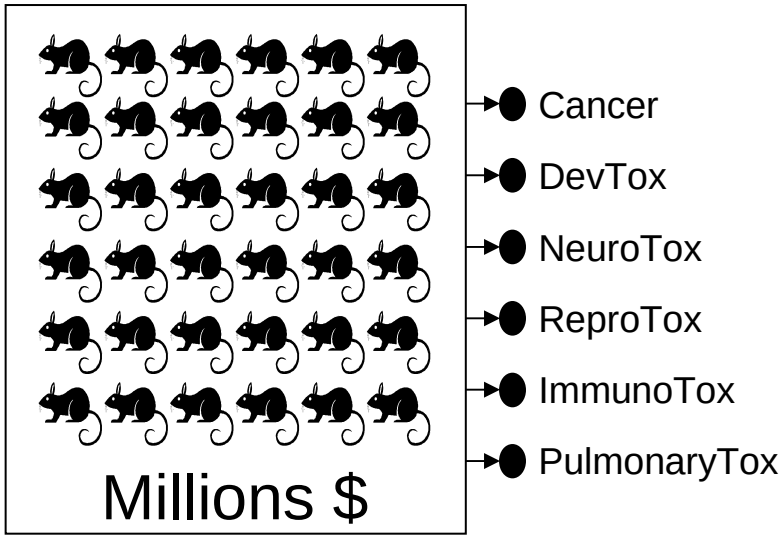


The Problem

Too Many Chemicals

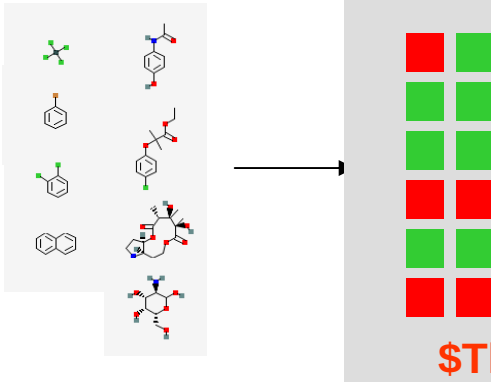


Too High a Cost



…and not enough data.

Future of Toxicity Testing



POLICYFORUM

TOXICOLOGY

Transforming Environmental Health Protection

We propose a shift from primarily in vivo animal studies to in vitro assays, in vivo assays with lower organisms, and computational modeling for toxicity assessments.

Francis S. Collins,[1*†] George M. Gray,[2*] John R. Bucher[3*]

In 2005, the U.S. Environmental Protection Agency (EPA), with support from the U.S. National Toxicology Program (NTP), funded a project at the National Research Council (NRC) to develop a long-range vision for toxicity testing and a strategic plan for implementing that vision. Both agencies wanted future toxicity testing and assessment paradigms to meet evolving regulatory needs. Challenges include the large numbers of substances that need to be tested and how to incorporate recent advances in molecular toxicology, computational sciences, and information technology; to rely increasingly on human as opposed to animal data; and to offer increased efficiency in design and costs (*1*–*5*). In response, the NRC Committee on Toxicity Testing and Assessment of Environmental Agents produced two reports that reviewed current toxicity testing, identified key issues, and developed a vision and implementation strategy to create a major shift in the assessment of chemical hazard and risk (*6*, *7*). Although the NRC reports have laid out a solid theoretical rationale, comprehensive and rigorously gathered data (and comparisons with historical animal data) will determine whether the hypothesized improvements will be realized in practice. For this purpose, NTP, EPA, and the National Institutes of Health Chemical Genomics Center (NCGC) (organizations with expertise in experimental toxicology, computational toxicology, and high-throughput technologies, respectively) have established a collaborative research program.

EPA, NCGC, and NTP Joint Activities

In 2004, the NTP released its vision and roadmap for the 21st century (*1*), which established initiatives to integrate high-throughput screening (HTS) and other automated screening assays into its testing program. In 2005, the EPA established the National Center for Computational Toxicology (NCCT). Through these initiatives, NTP and EPA, with the NCGC, are promoting the evolution of toxicology from a predominantly observational science at the level of disease-specific models in vivo to a predominantly predictive science focused on broad inclusion of target-specific, mechanism-based, biological observations in vitro (*1*, *4*) (see figure, below).

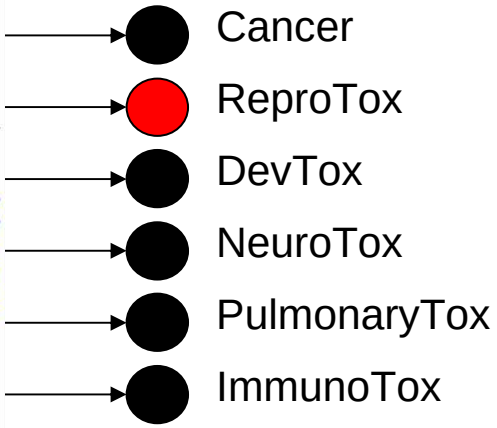
Toxicity pathways. In vitro and in vivo tools are being used to identify cellular responses after chemical exposure expected to result in adverse health effects (*7*). HTS methods are a primary means of discovery for drug development, and screening of >100,000 compounds per day is routine (*8*). However, drug-discovery HTS methods traditionally test compounds at one concentration, usually between 2 and 10 μM, and tolerate high false-negative rates. In contrast, in the EPA, NCGC, and NTP combined effort, all compounds are tested at as many as 15 concentrations, generally ranging from ~5 nM to ~100 μM, to generate a concentration-response curve (*9*). This approach is highly reproducible, produces significantly lower false-positive and false-negative rates than the traditional HTS methods (*9*), and facilitates multiassay comparisons. Finally, an informatics platform has been built to compare results among HTS screens; this is being expanded to allow comparisons with historical toxicologic NTP and EPA data (http://ncgc.nih.gov/pub/openhts). HTS data collected by EPA and NTP, as well as by the NCGC and other Molecular Libraries Initiative centers (http://mli.nih.gov/), are being made publicly available through Web-based databases [e.g., PubChem (http://pubchem.ncbi.nlm.nih.gov)]. In addition,

Transforming toxicology. The studies we propose will test whether high-throughput and computational toxicology approaches can yield data predictive of results from animal toxicity studies, will allow prioritization of chemicals for further testing, and can assist in prediction of risk to humans.

[1]Director, National Human Genome Research Institute (NHGRI), National Institutes of Health, Bethesda, MD 20892; [2]Assistant Administrator for the Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC 20460; [3]Associate Director, U.S. National Toxicology Program, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC 27709, USA.

*The views expressed here are those of the individual authors and do not necessarily reflect the views and policies of their respective agencies.

†Author for correspondence. E-mail: francisc@mail.nih.gov

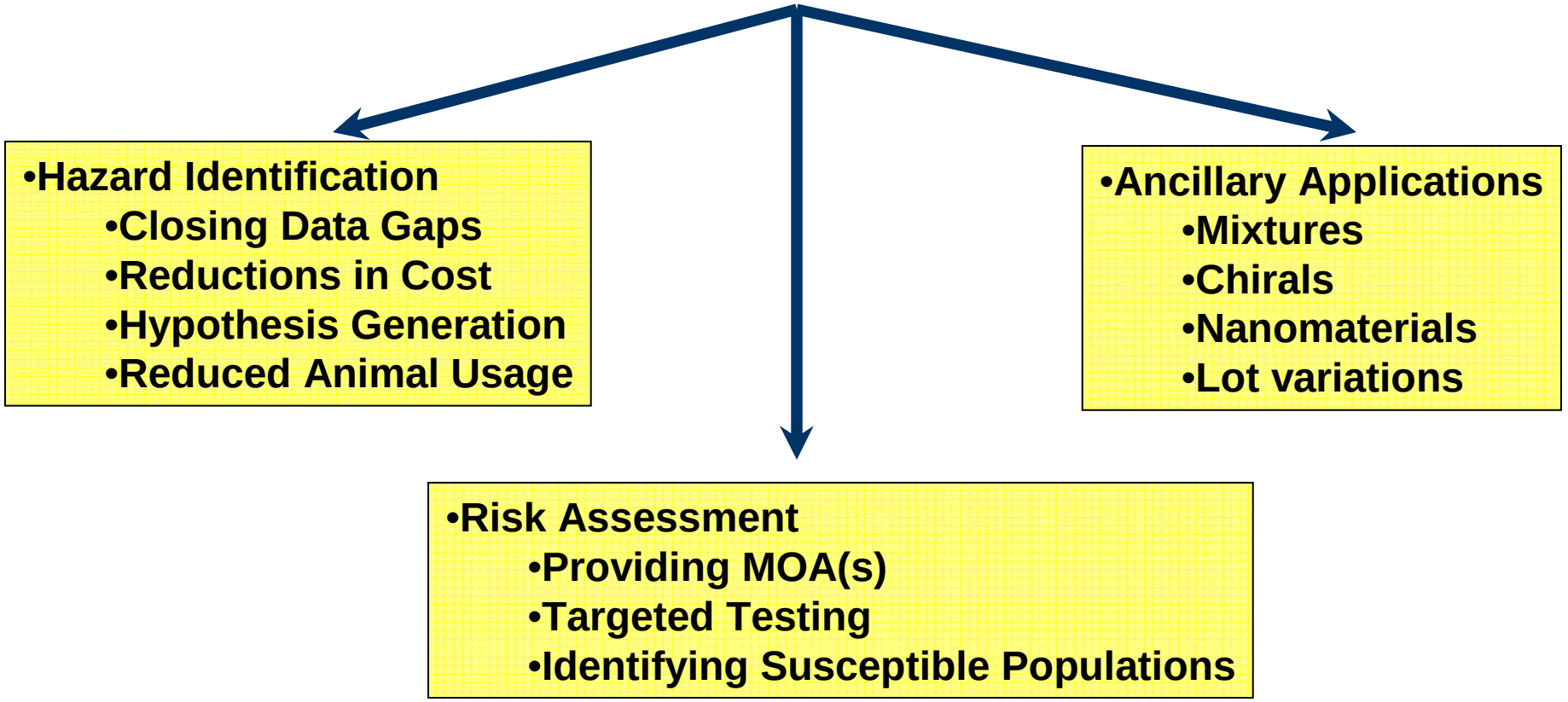


EPAs Contribution: The ToxCast Research Program

Key Challenges

- Find the Toxicity Pathways
  - Hepato vs developmental
- Obtain HTS Assays for Them
  - Including metabolic capability
- Screen Chemical Libraries
  - Coverage of p-chem properties
- Link Results to in vivo Effects
  - Gold standard and dosimetry

Implications for Success



Phased Development of ToxCast

Phase	Number of Chemicals	Chemical Criteria	Purpose	Number of Assays	Cost per Chemical	Target Date
I	320	Data Rich (pesticides)	Signature Development	>400	$20k	FY07-08
IIa	>300	Data Rich Chemicals	Validation	>400	$15-20k	FY09
IIb	>100	Known Human Toxicants	Extrapolation	>400	$15-20k	FY09
IIc	>300	Expanded Structure and Use Diversity	Extension	>400	$15-20k	FY10
III	Thousands	Data poor	Prediction and Prioritization	???	$10-15k	FY11-12

- Affordable science-based system for categorizing chemicals
- Increasing confidence as database grows
- Identifies potential mechanisms of action
- Refines and reduces animal use for hazard ID and risk assessment

The ToxCast_320

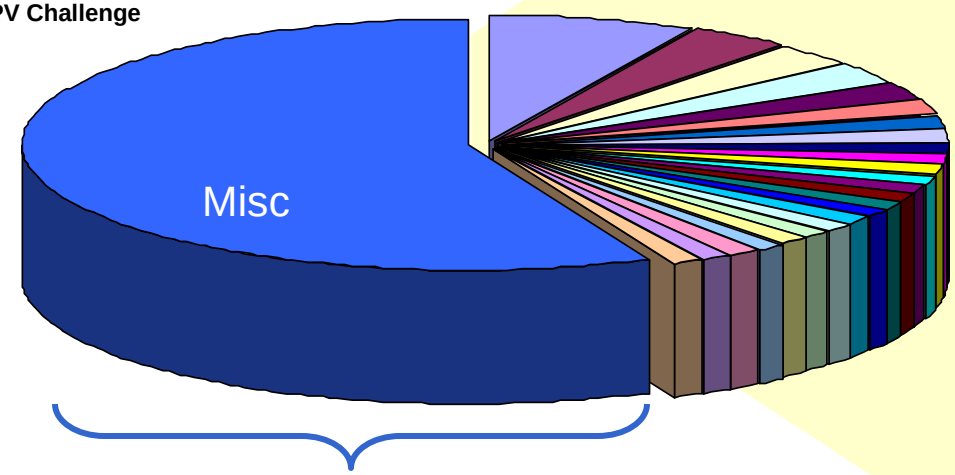
309 Unique Structures

Replicates for QC

291 Pesticide Actives
9 Industrial Chemicals
8 Metabolites

56/73 Proposed Tier 1 EDSP

14 HPV
11 HPV Challenge



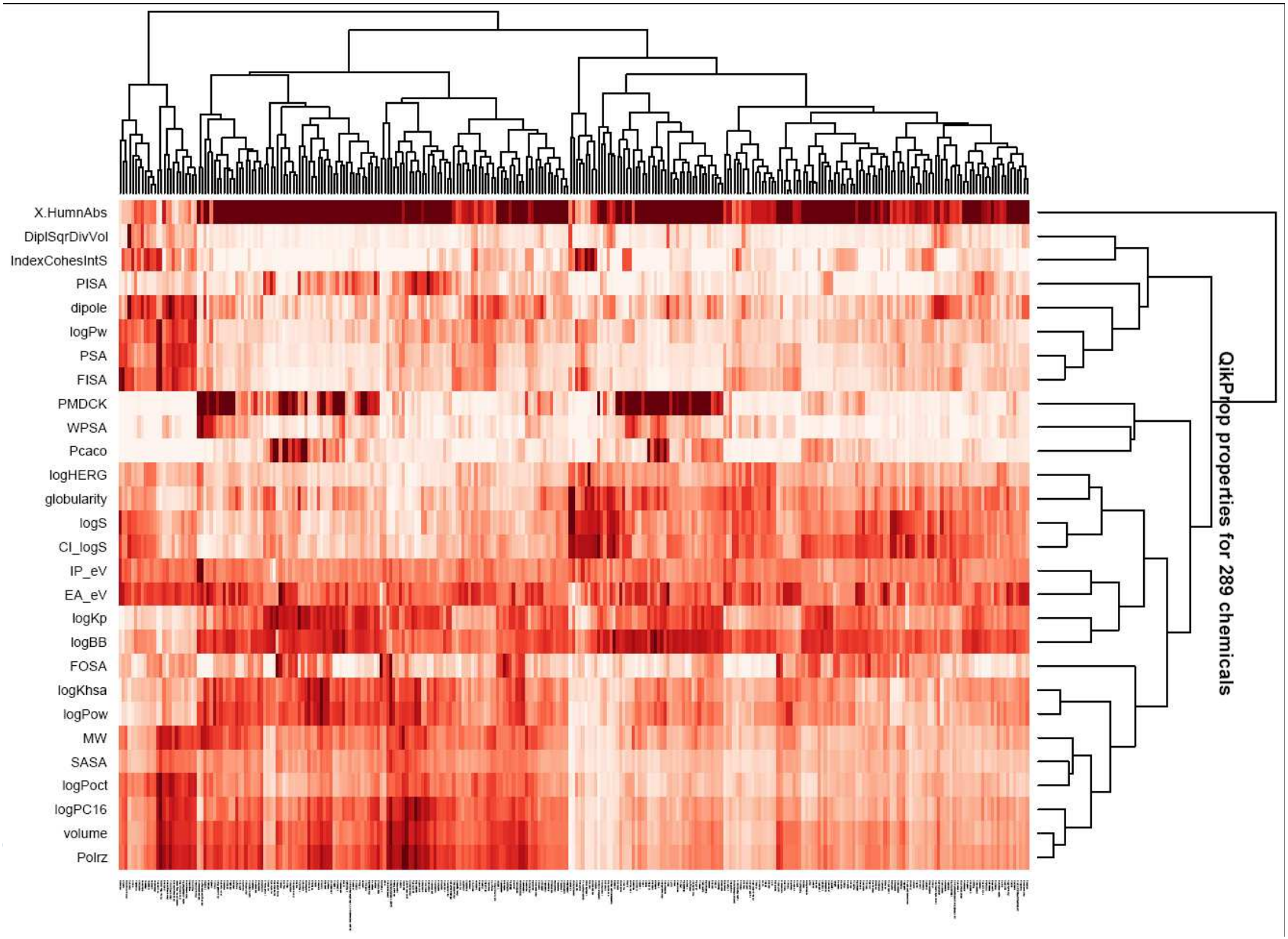
Misc MOA classes with
3 or fewer representatives

Classes with
> 3 chemicals

- **Acetylcholine esterase inhibitors**
- **conazole fungicides**
- **Sodium channel modulators**
- **pyrethroid ester insecticides**
- **organothiophosphate acaricides**
- **dinitroaniline herbicides**
- **pyridine herbicides**
- **thiocarbamate herbicides**
- **imidazolinone herbicides**
- **organophosphate insecticides**
- **phenyl organothiophosphate insecticides**
- **aliphatic organothiophosphate insecticides**
- **amide herbicides**
- **aromatic fungicides**
- **chloroacetanilide herbicides**
- **chlorotriazine herbicides**
- **growth inhibitors**
- **organophosphate acaricides**
- **oxime carbamate insecticides**
- **phenylurea herbicides**
- **pyrethroid ester acaricides**
- **strobilurin fungicides**
- **unclassified acaricides**
- **unclassified herbicides**

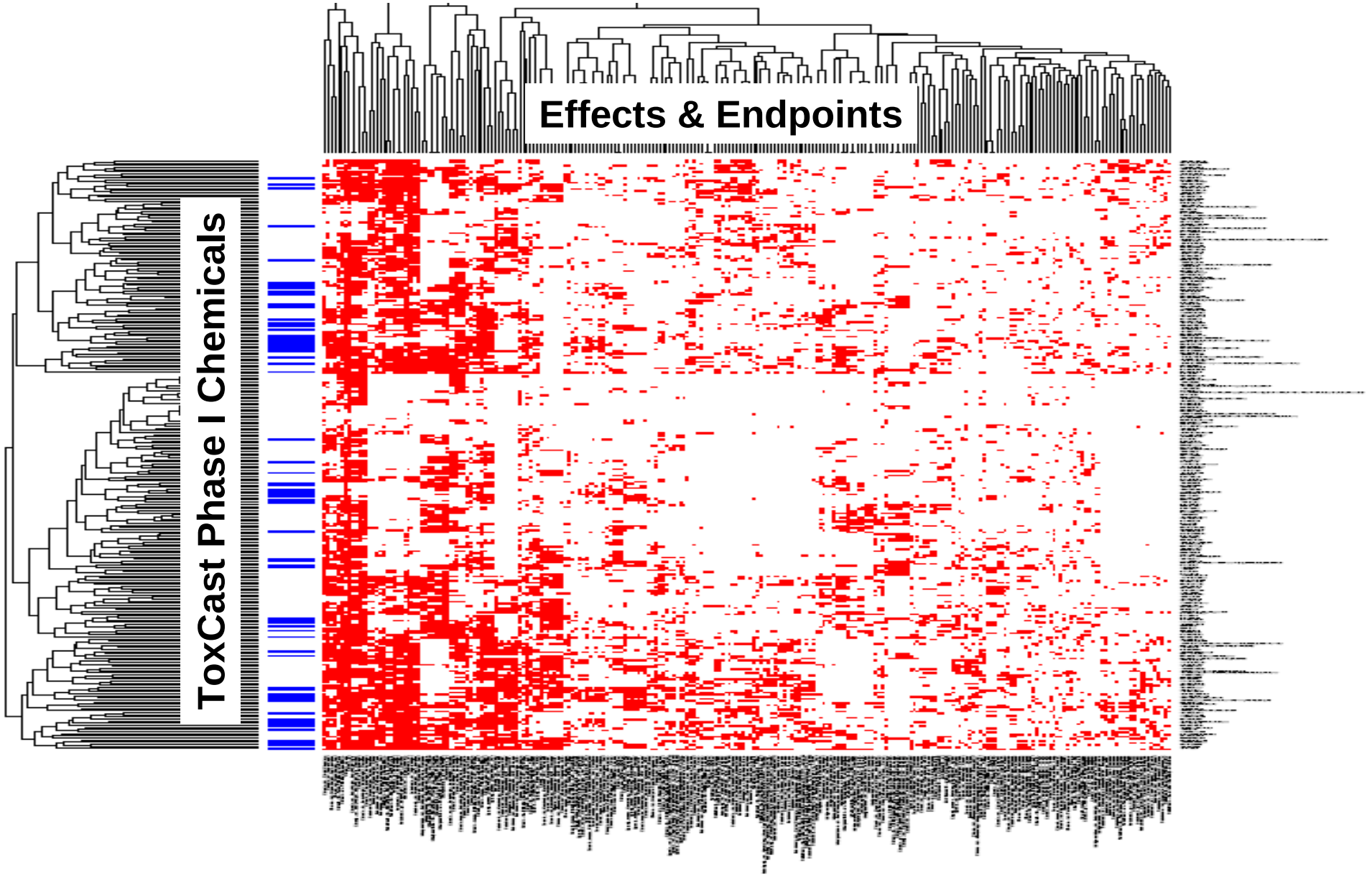
Classification based on OPPIN

Physical-Chemical Properties

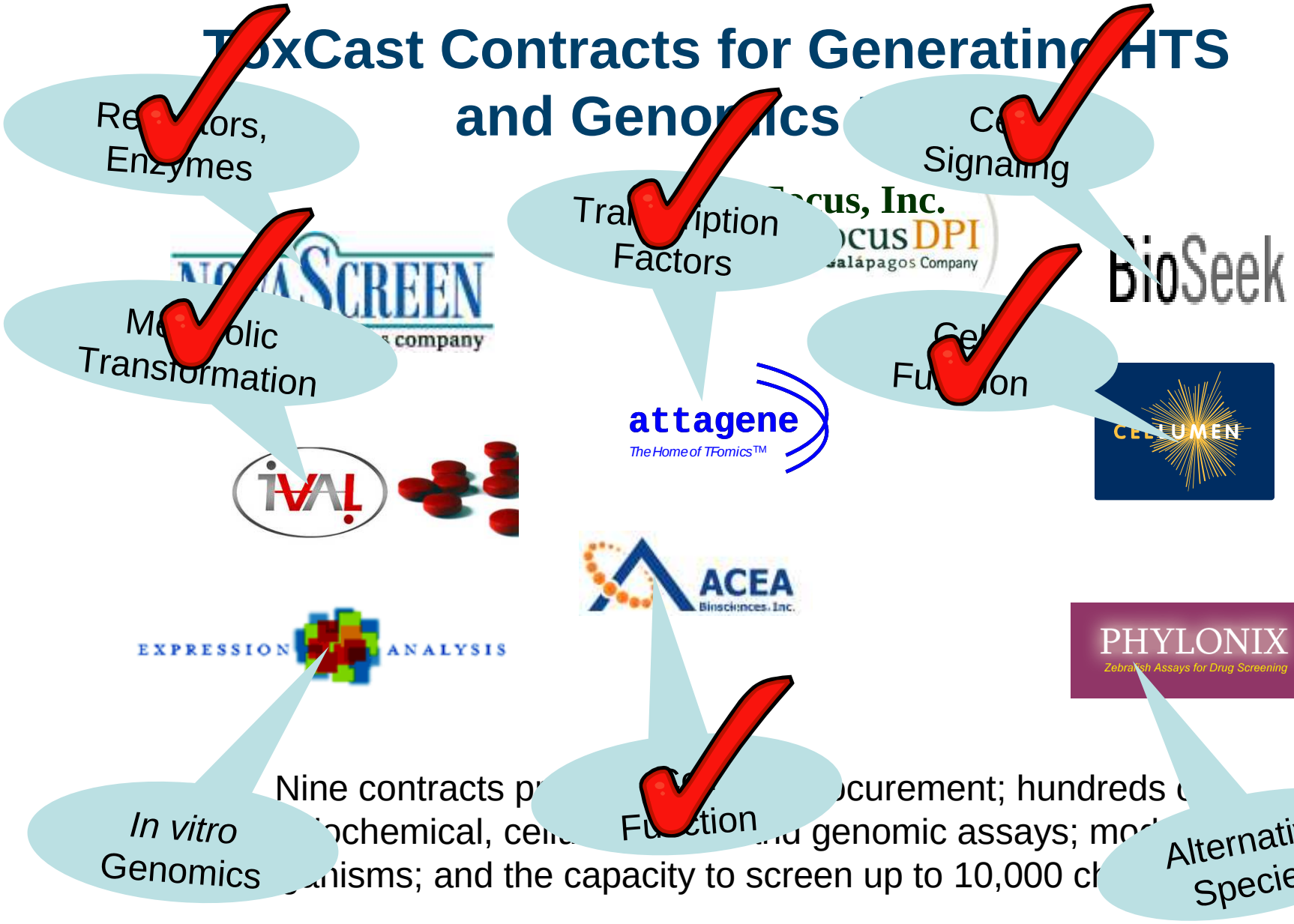


$400 Million Dollars Worth of *In Vivo* Chronic/Cancer Bioassay Effects and Endpoints

EPA United States Environmental Protection Agency



ToxCast Contracts for Generating HTS and Genomics



Nine contracts p… procurement; hundreds o… biochemical, cell… and genomic assays; mo… organisms; and the capacity to screen up to 10,000 ch…

Evolution of Phase I

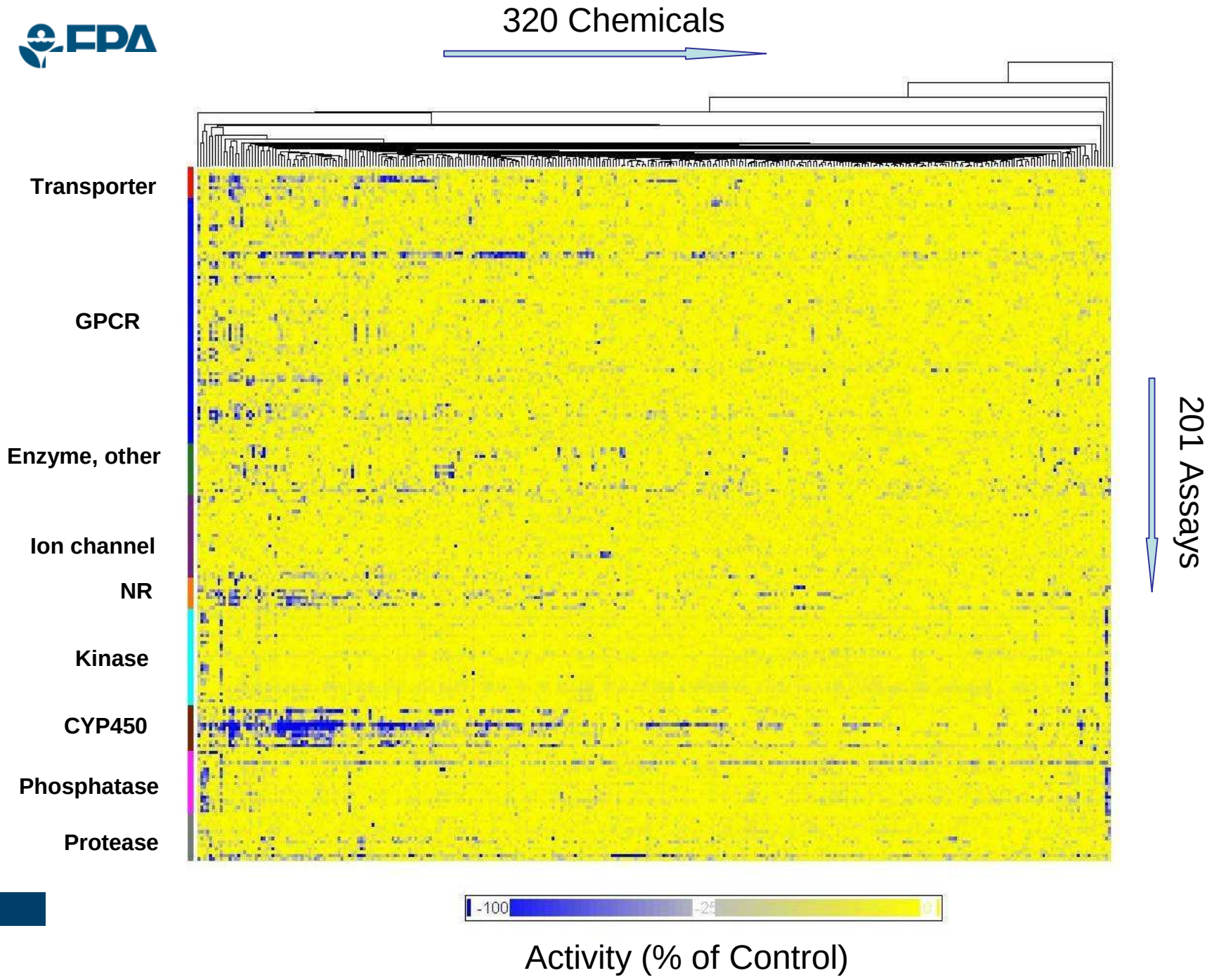
- **ToxCast 1.0 (April, 2007)**
  - *Enzyme inhibition/receptor binding HTS (Novascreen)*
  - *NR/transcription factors (Attagene, NCGC)*
  - *Cellular impedance (ACEA)*
  - *Complex cell interactions (BioSeek)*
  - *Hepatocelluar HCS (Cellumen)*
  - *Hepatic, renal and airway cytotoxicity (IVAL)*
  - *In vitro hepatogenomics (IVAL, Expression Analysis)*
  - *Zebrafish developmental toxicity (Phylonix)*
- **ToxCast 1.1 (January, 2008)**
  - *Neurite outgrowth HCS (NHEERL)*
  - *Cell proliferation (NHEERL)*
  - *Zebrafish developmental toxicity (NHEERL)*
- **ToxCast 1.2 (March, 2008)**
  - *Organ culture: liver, kidney, lung (Hamner Institutes)*
  - *HTS Genotoxicity (Gentronix)*
  - *Toxicity and signaling pathways (Invitrogen)*
  - *NR Activation and translocation (CellzDirect)*
  - *3D Cellular microarray with metabolism (Solidus)*
  - *C. elegans (NIEHS)*
  - *Functional markers from microscale cultured hepatocytes (MIT)*

9 Assay Sources & 412 Endpoints

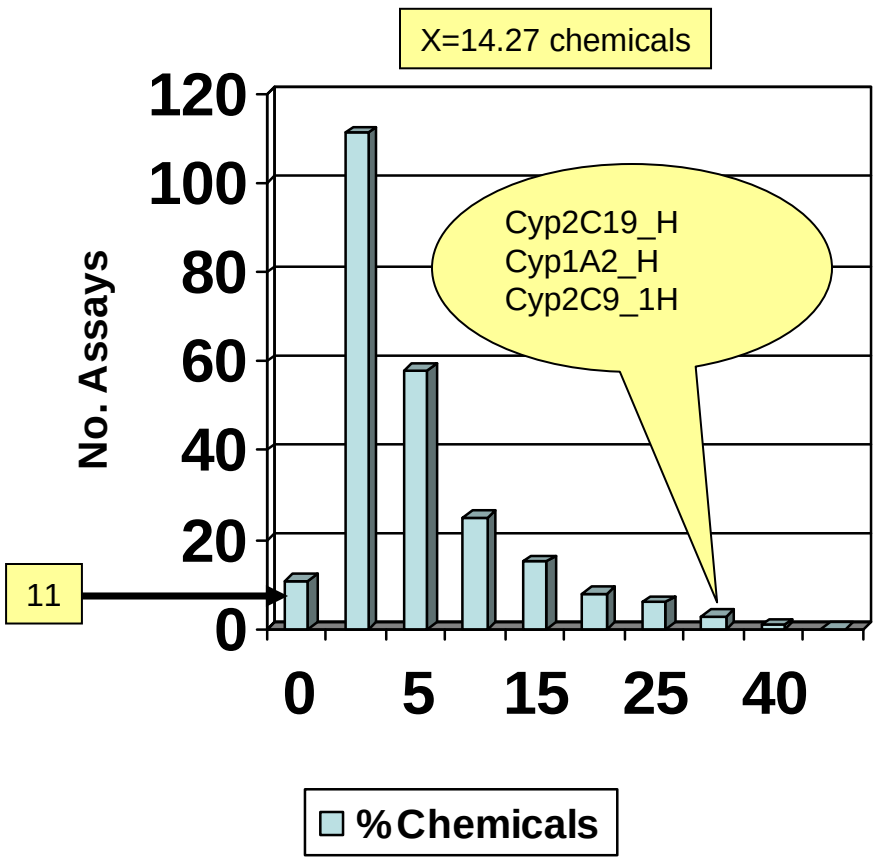
+3 Assay Sources & 16 Endpoints

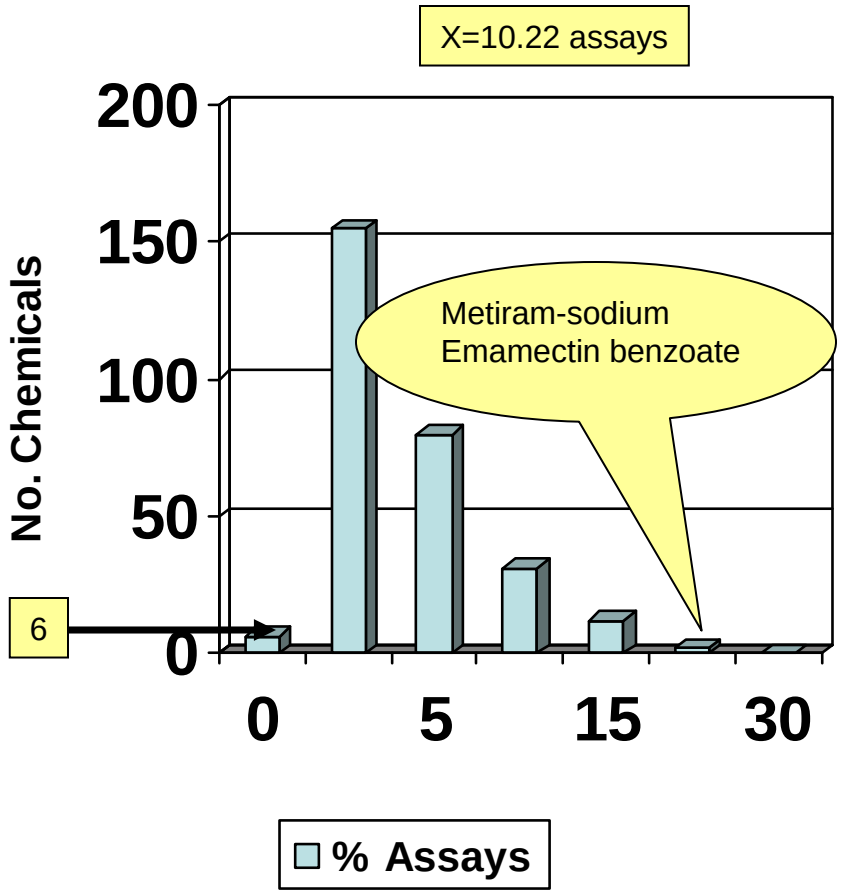
+7 Assay Sources & 123 Endpoints

19 Assay Sources, 551 Endpoints

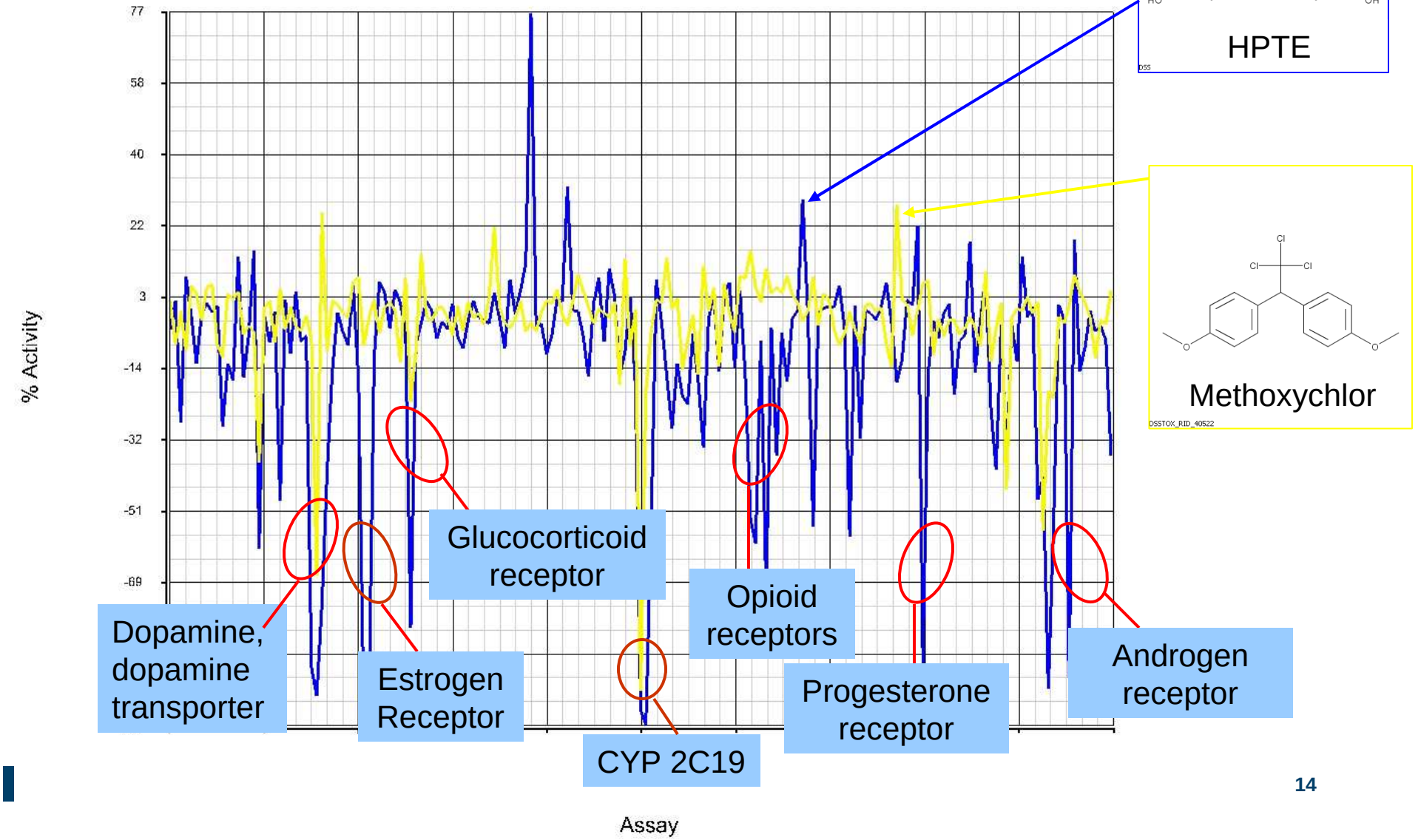

320 Chemicals
Transporter
GPCR
Enzyme, other
Ion channel
NR
Kinase
CYP450
Phosphatase
Protease
201 Assays
-100
Activity (% of Control)

NovaScreen Descriptive Statistics (30% Cutoff)





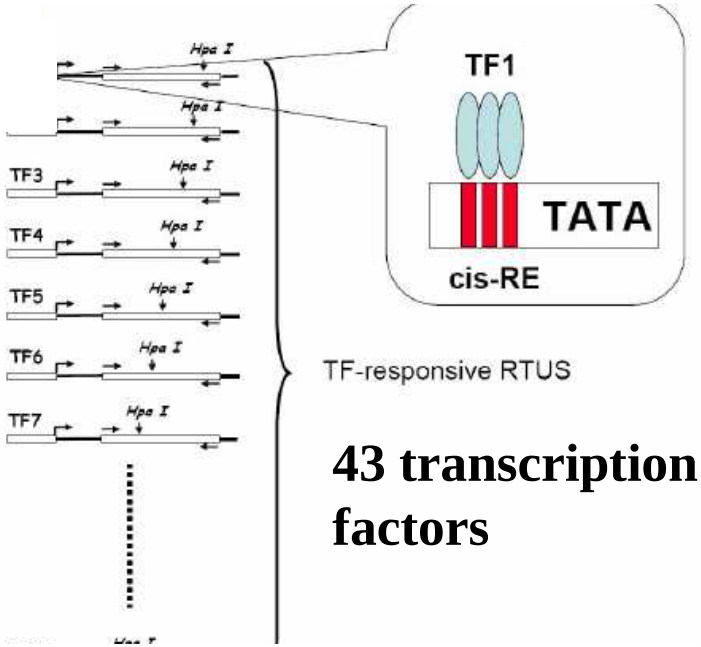
Activity Spectrum (Novascreen)



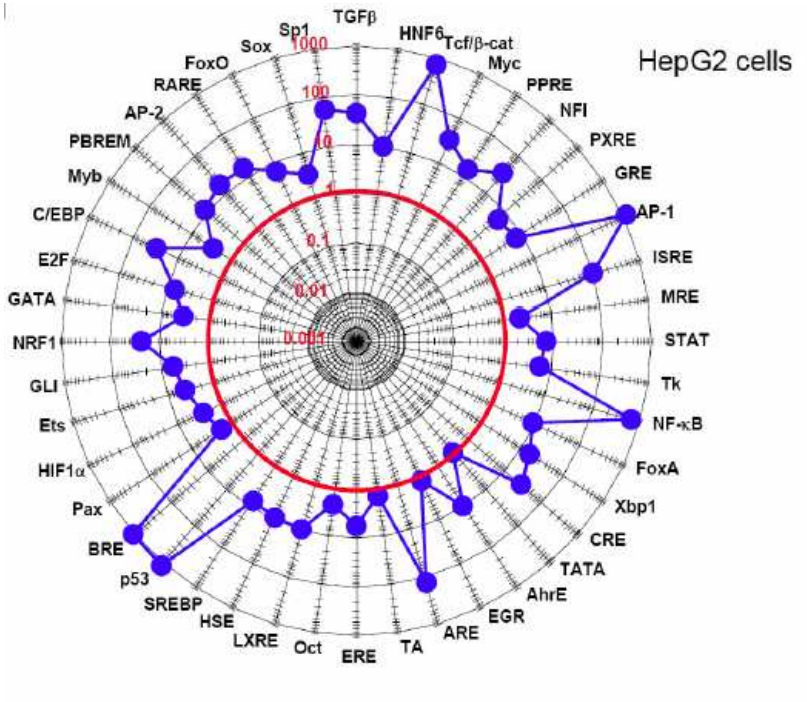
Transcription Factor Activity Profiling

attagene
The Home of TFomics™

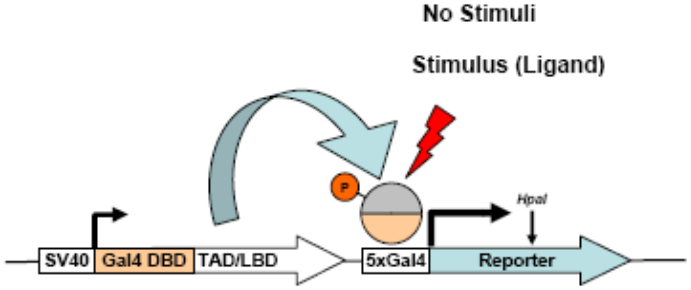
Cis-Factorial™ Biosensors



43 transcription factors

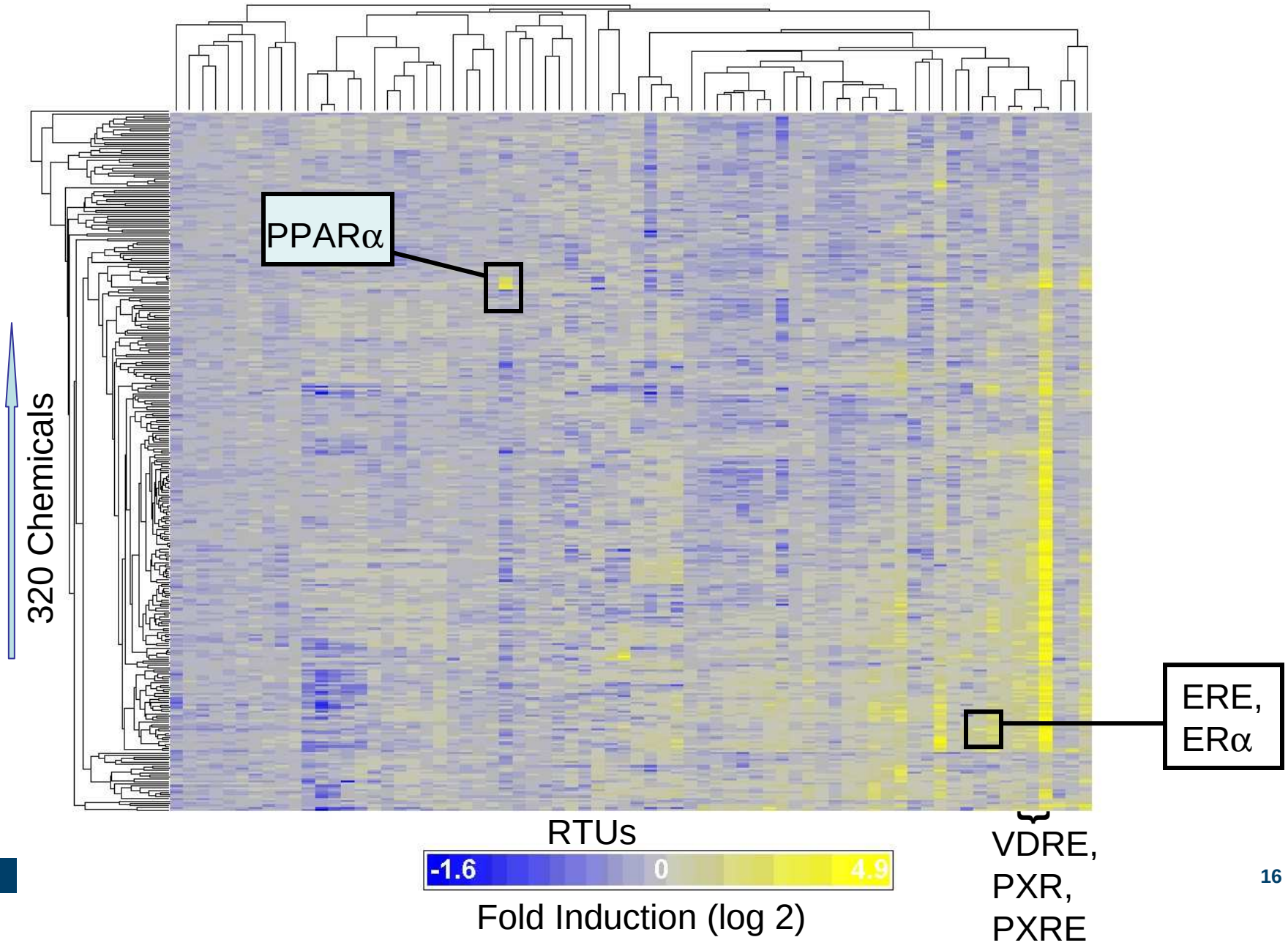


Trans-Factorial™ Biosensors



24 nuclear receptors

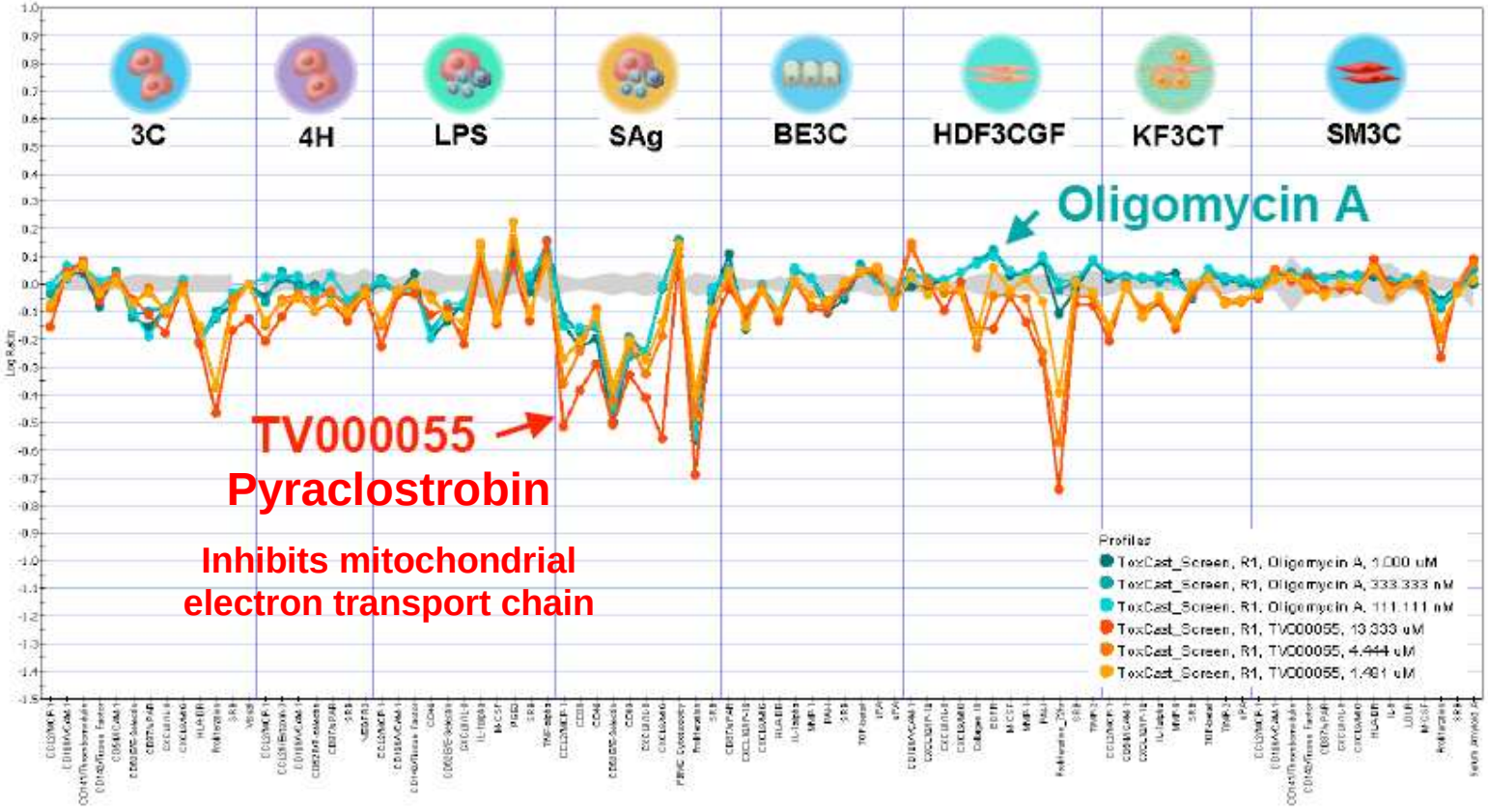
Hierachical Cluster Attagene Results



BioMAP Systems for EPA ToxCast

System	Cell Types	Environment	Readout Parameters
3C 	Endothelial cells	IL-1β+TNF-α+IFN-γ	MCP-1, VCAM-1, ICAM-1, Thrombomodulin, Tissue Factor, E-selectin, uPAR, IL-8, MIG, HLA-DR, Proliferation, Vis., SRB (13)
4H 	Endothelial cells	IL-4+histamine	VEGFRII, P-selectin, VCAM-1, uPAR, Eotaxin-3, MCP-1, SRB (7)
LPS 	Peripheral blood mononuclear cells + Endothelial cells	TLR4	CD40, VCAM-1,Tissue Factor, MCP-1, E-selectin, IL-1α, IL-8, M-CSF, TNF-α, PGE2, SRB (11)
SAg 	Peripheral blood mononuclear cells + Endothelial cells	TCR	MCP-1, CD38, CD40, CD69, E-selectin, IL-8, MIG, PBMC Cytotox., SRB, Proliferation (10)
BE3C 	Bronchial epithelial cells	I IL-1β+TNF-α+IFN-γ	uPAR, IP-10, MIG, HLA-DR, IL-1α, MMP-1, PAI-1, SRB, TGF-b1, tPA, uPA (11)
HDF3CGF 	Fibroblasts	IL-1β+TNF-α+IFN-γ +bFGF+EGF+PDGF-BB	VCAM-1, IP-10, IL-8, MIG, Collagen III, M-CSF, MMP-1, PAI-1, Proliferation, TIMP-1, EGFR, SRB (12)
KF3CT 	Keratinocytes + Fibroblasts	IL-1β+TNF-α+IFN-γ+TGF-β	MCP-1, ICAM-1, IP-10, IL-1α, MMP-9, TGF-β1, TIMP-2, uPA, SRB (9)
SM3C 	Vascular smooth muscle cells	IL-1β+TNF-α+IFN-γ	MCP-1, VCAM-1, Thrombomodulin, Tissue Factor, IL-6, LDLR, SAA, uPAR, IL-8, MIG, HLA-DR, M-CSF, Prolif., SRB (14)

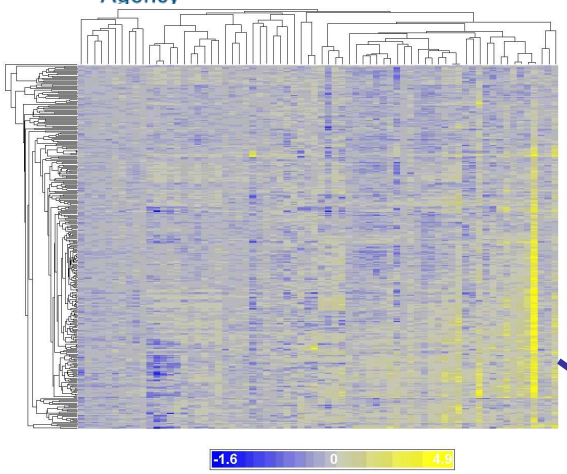
BioMAP Profiles of Oligomycin A and TV000055



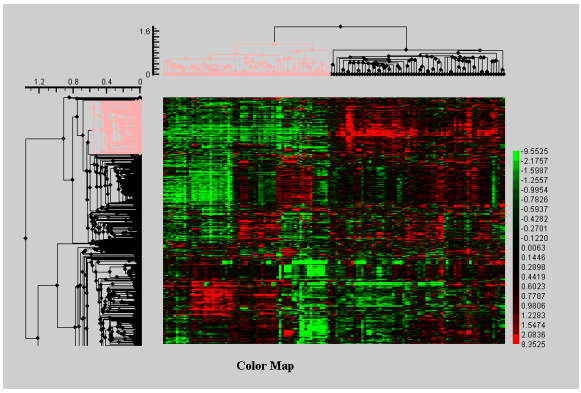
- **Oligomycin A is an inhibitor of mitochondrial ATPase**
- **Similarity suggests inhibition of mitochondrial function by TV000055**
  - (TV00005 is most similar to Complex I inhibitors)

Correlating HTS to Toxicity

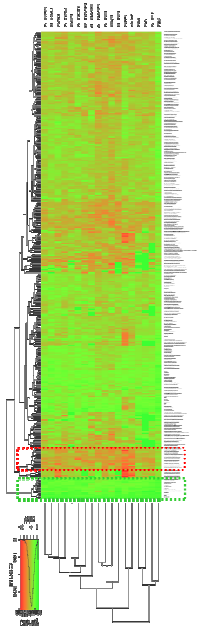
United States Environmental Protection Agency



Cellular Assays

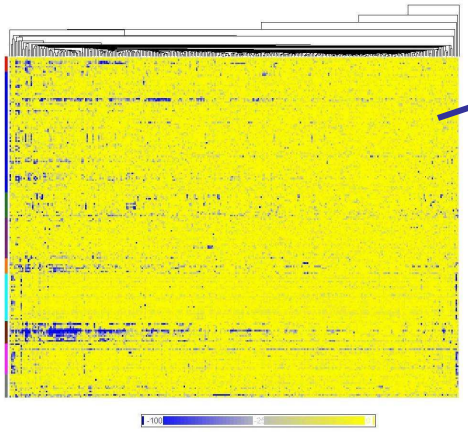


Physical chemical properties

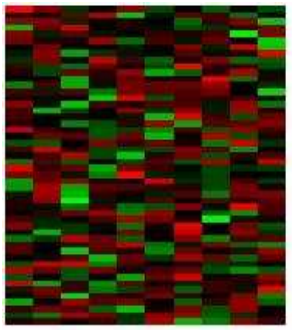


In silico Predictions

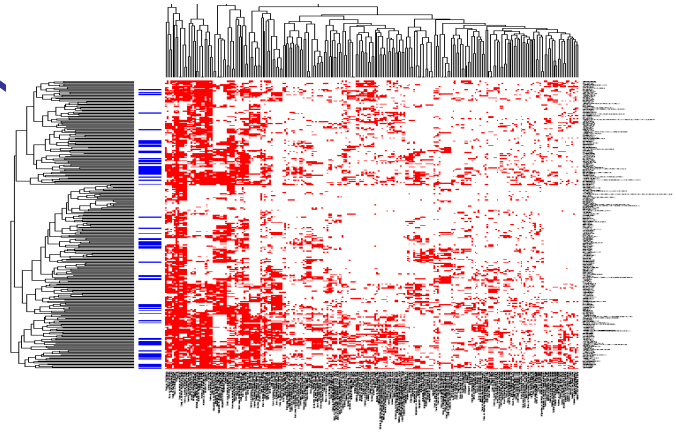
Profile Matching



Biochemical Assays



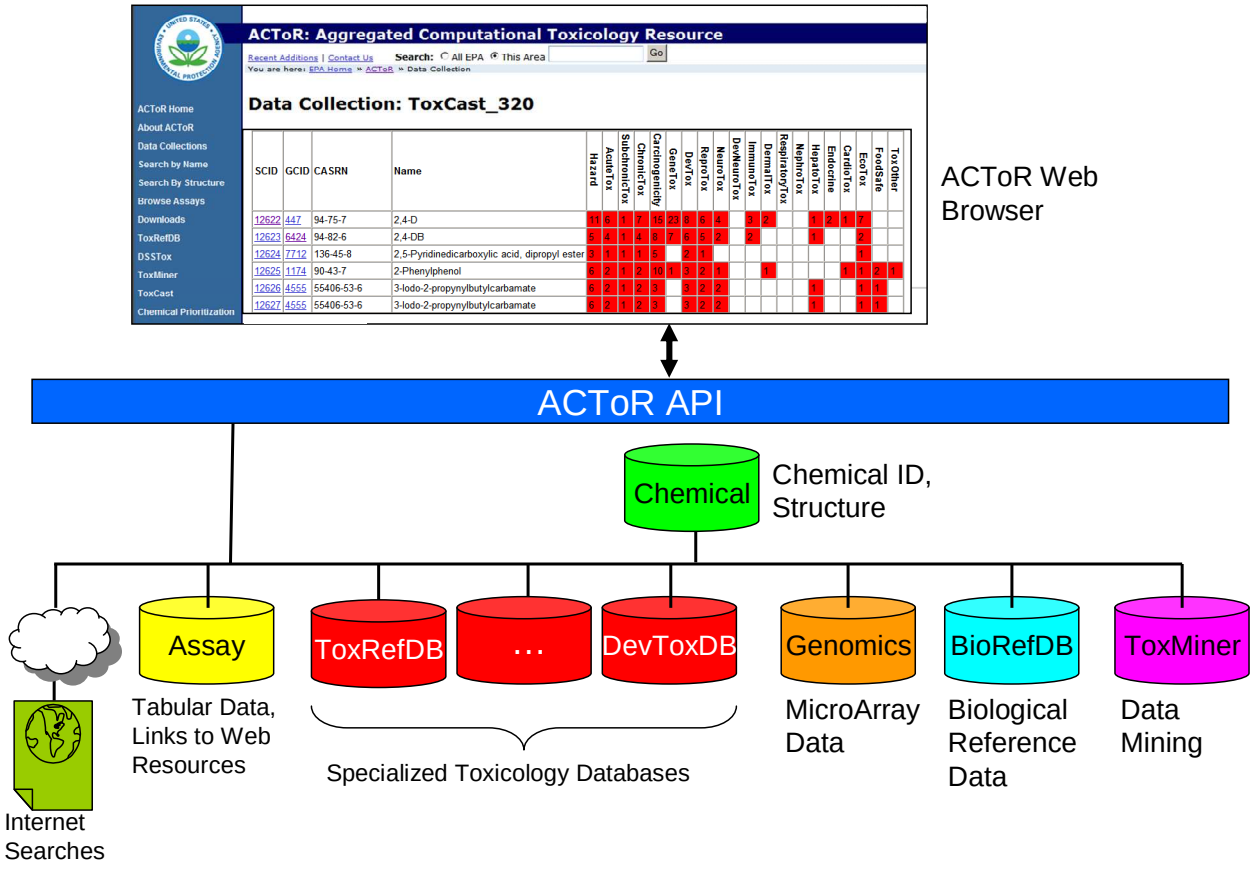
Genomic Signatures



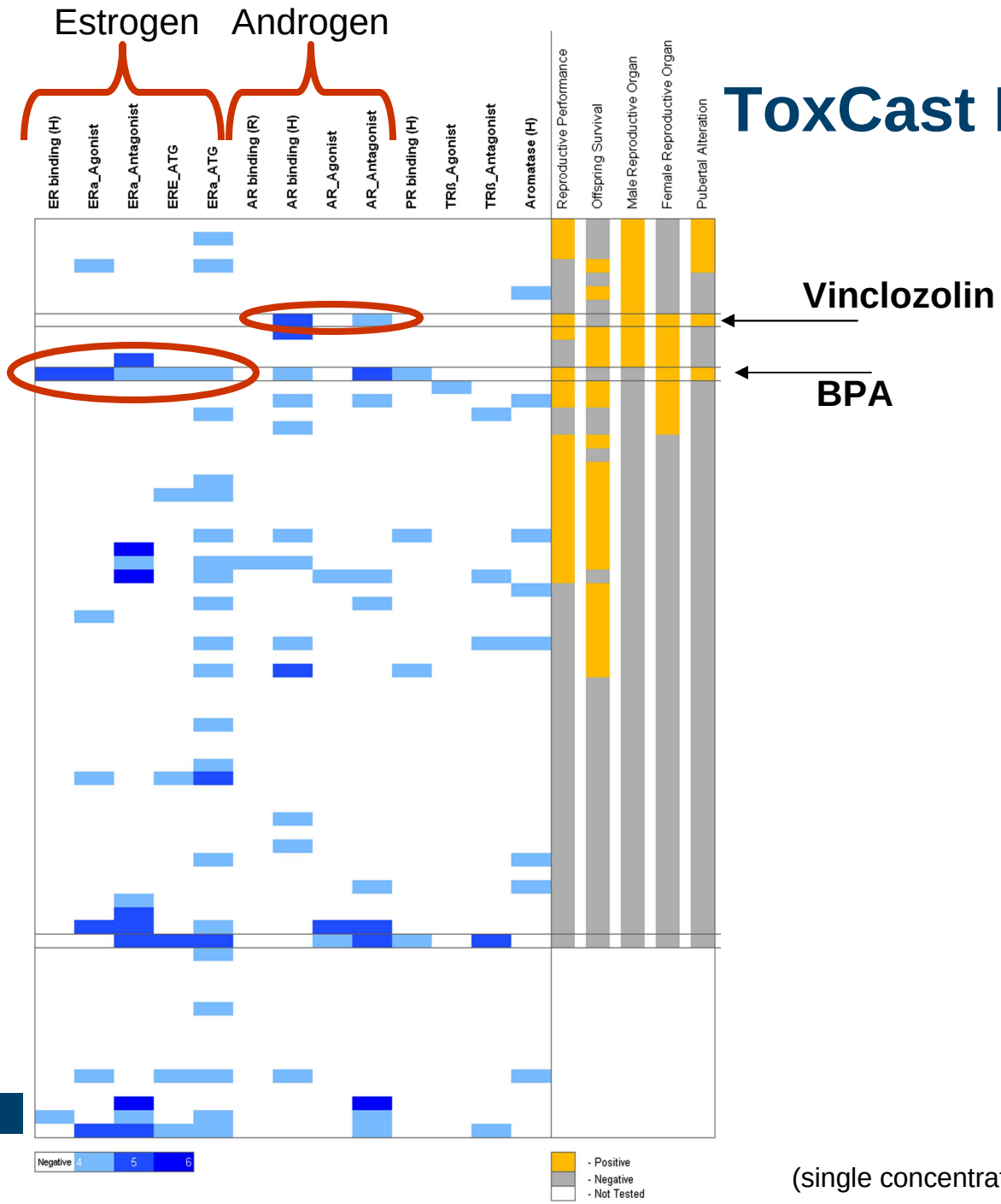
Toxicology Endpoints

United States Environmental Protection Agency

ACToR: Aggregated Computational Toxicology Resource



ToxCast Endocrine Profiling



HTS Data from receptor binding (Novascreen), single gene reporter (NCGC) response element activation (Attagene) for the proposed Endocrine Disruptor Screening Program Priority Chemicals contained in the ToxCast 320

(single concentration only for binding and transcription factors)

Moving Forward

- Completion of Data Acquisition and Data Mining for Phase I
- Publication and Public Release of all Data
- OECD Molecular Screening Initiative (June, Bilthoven)
- Data Summit, Fall/Winter 2008
- Tox21 MOU partnership with NTP/NIEHS and NCGC/NHGRI
  - Four Working Groups
  - Total of ~7000 chemicals for screening
  - Subset to feed Phase II of ToxCast
- Communities of Practice – Prioritization (Dix), Exposure (Hubal)
- EPA Research Strategy

Toxicity Pathways in Prioritization ⟷ Toxicity Pathways in Risk Assessment ⟷ Institutional Transition

The ToxCast Team

ToxCast Website: www.epa.gov/ncct/toxcast

United States Environmental Protection Agency

National Center for Computational Toxicology

Contact Us Search: All EPA This Area Go

You are here: EPA Home » National Center for Computational Toxicology » ToxCast™ Program

The EPA Web site will be unavailable on Sunday, March 2, 2008 from 8:00 pm until 10:00 pm ET.

ToxCast™ Program

Predicting Hazard, Characterizing Toxicity Pathways, and Prioritizing the Toxicity Testing of Environmental Chemicals

Introduction

ToxCast™ Navigation

- Introduction
- ToxCast™ Chemicals
- ToxCast™ Assays
- ToxCast™ Information Management
- ToxCast™ Partnerships
- ToxCast™ Contractors
- ToxCast™ Presentations
- ToxCast™ Publications
- ToxCast™ News

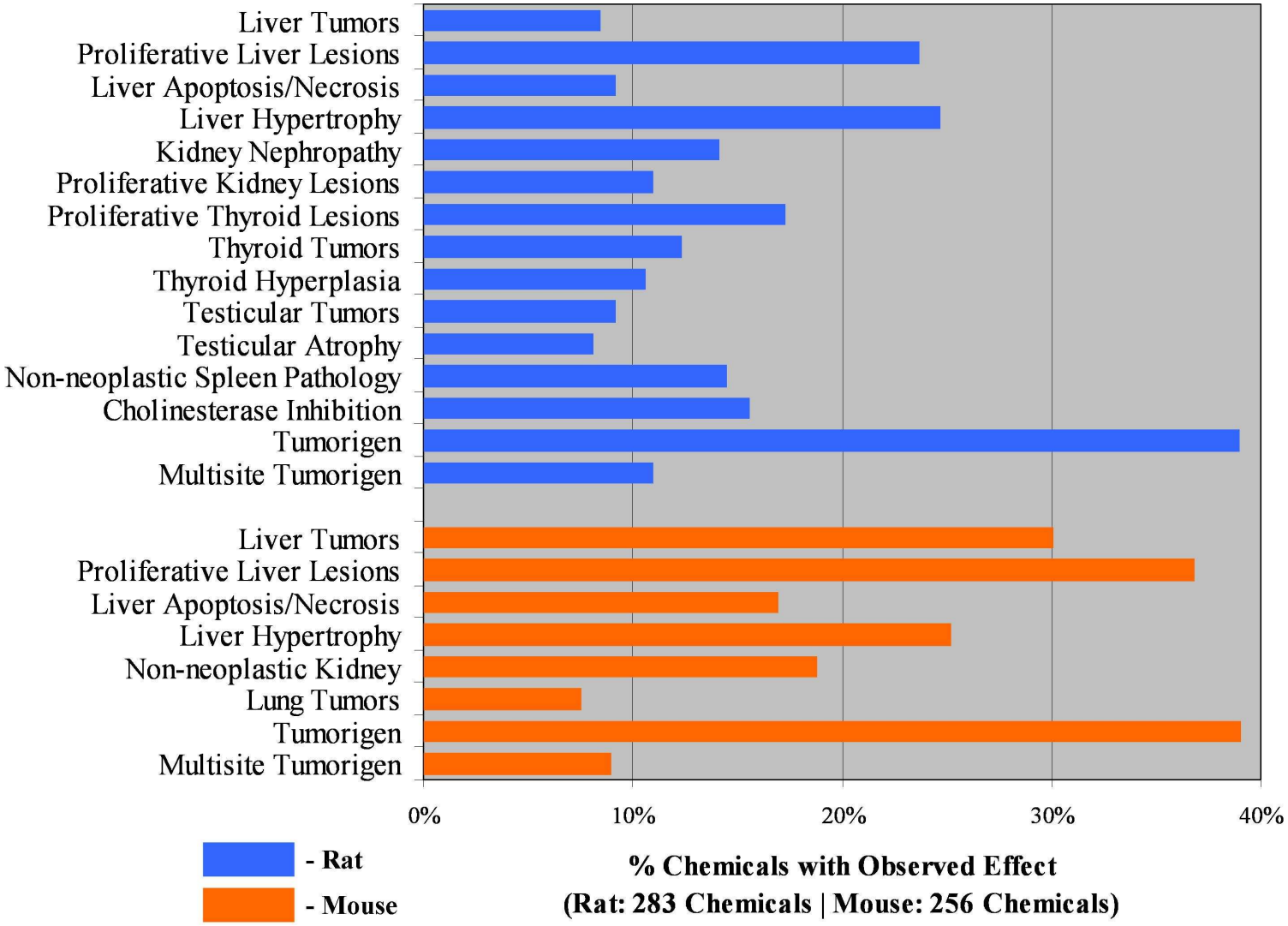
In 2007, EPA launched ToxCast™ in order to develop a cost-effective approach for prioritizing the toxicity testing of large numbers of chemicals in a short period of time. Using data from state-of-the-art high throughput screening (HTS) bioassays developed in the pharmaceutical industry, ToxCast™ is building computational models to forecast the potential human toxicity of chemicals. These hazard predictions will provide EPA regulatory programs with science-based information helpful in prioritizing chemicals for more detailed toxicological evaluations, and lead to more efficient use of animal testing.

In its first phase, ToxCast™ is profiling over 300 well-characterized chemicals (primarily pesticides) in over 400 HTS endpoints. These endpoints include biochemical assays of protein function, cell-based transcriptional reporter assays, multi-cell interaction assays, transcriptomics on primary cell cultures, and developmental assays in zebrafish embryos. Almost all of the compounds being examined in Phase 1 of ToxCast™ have been tested in traditional toxicology tests, including developmental toxicity, multi-generation studies, and sub-chronic and chronic rodent bioassays. ToxRefDB, a relational database being created to house this information, will contain nearly $1B worth of toxicity studies in animals when completed. ToxRefDB is integrated into a more comprehensive data management system developed by NCCT called ACToR (Aggregated Computational Toxicology Resource), that manages the large-scale datasets of ToxCast™. ACToR is comprised of several independent data repositories linked to a common database of chemical structures and properties, and to tools for development of predictive HTS and genomic bioactivity signatures that strongly correlate with specific toxicity endpoints from ToxRefDB. These ToxCast™ signatures will be defined and evaluated by their ability to predict outcomes from existing mammalian toxicity testing, and identify toxicity pathways that are relevant to human health effects.

The second phase of ToxCast™ will screen additional compounds representing broader chemical structure and use classes, in order to evaluate the predictive bioactivity signatures developed in Phase I. Following successful conclusion of Phases I and II, ToxCast™ will provide EPA regulatory programs an efficient tool for rapidly and efficiently screening compounds and prioritizing further toxicity testing.

United States
Environmental Protection
Agency

Common Phenotypes in Chronic Rodent Studies



Comparing Activities by Chemical Class

Conazole Fungicides vs. NovaScreen Assays

NAME	CYP2C19	CYP2C9	CYP3A1	Dopamine Transporter (Human)	CYP2D2	Androgen Receptor	Dopamine Transporter (Rat)	CYP2B6	CYP2D1	CYP3A4	Progesterone Receptor	Benzodiazepine Receptor
Cyproconazole	1	1	1	1	1	0	1	0	0	1	0	0
Difenoconazole	1	1	1	1	1	0	0	1	1	0	0	0
Diniconazole	1	1	1	0	1	0	0	0	1	1	1	0
Fenbuconazole	1	1	0	0	0	0	0	0	0	1	0	0
Flusilazole	1	1	1	0	1	1	0	1	1	NA	1	1
Hexaconazole	1	1	1	1	1	0	1	1	1	NA	1	0
Imazalil	1	1	1	1	1	1	1	1	1	1	1	1
Myclobutanil	1	1	1	1	0	0	0	0	0	NA	0	0
Paclobutrazol	1	0	1	1	0	1	1	0	1	1	0	0
Prochloraz	1	1	1	1	1	1	1	1	1	NA	1	1
Propiconazole	1	1	1	0	0	0	0	1	0	NA	0	1
Tetraconazole	1	1	1	0	1	1	0	1	0	1	1	0
Triadimefon	1	1	0	1	1	1	1	0	0	1	0	1
Triadimenol	1	0	0	1	0	1	1	0	0	0	0	0
Triflumizole	1	1	1	1	1	1	0	1	1	1	1	1
Triticonazole	1	1	1	1	0	1	1	0	0	NA	0	0
Totals	**16**	**14**	**13**	**11**	**10**	**9**	**8**	**8**	**8**	**8**	**7**	**6**